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**Marek, Ales; Baun, Michael ; Bruun, Susanne W. ; Pedersen, Martin Holst Friborg**

*Publication date:*  
2012

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### *Citation (APA):*

Marek, A., Baun, M., Bruun, S. W., & Pedersen, M. H. F. (2012). *Tritium labelling of peptides using synthetic incorporated diiodinated tyrosine amino acid residues as precursor*. Abstract from 11th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Heidelberg, Germany.

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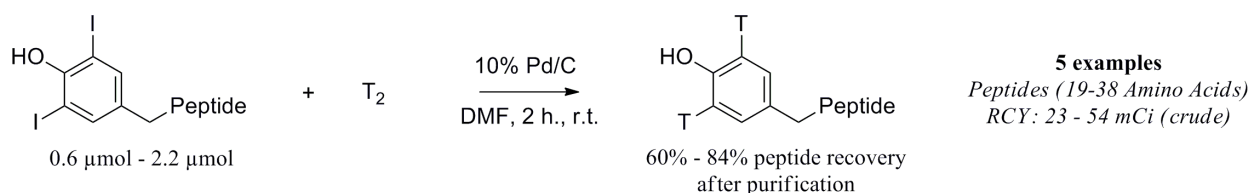
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# Tritium labelling of peptides using synthetic incorporated diiodinated tyrosine amino acid residues as precursor

Aleš Marek<sup>a†</sup>, Michael Baun<sup>b</sup>, Susanne W. Bruun<sup>c</sup> and Martin H. F. Pedersen<sup>a</sup>

<sup>a</sup>Technical University of Denmark, DTU Nutech, The Hevesy Laboratory, Frederiksborgvej 399, 4000 Roskilde, Denmark. <sup>b</sup>Danish Headache Center, Department of Neurology, Glostrup Hospital, Faculty of Health Sciences, University of Copenhagen, Denmark. <sup>c</sup>The Bartholin Institute, Rigshospitalet, Copenhagen Biocenter.  
E-mail: mhfp@dtu.dk



We have employed standard tritiodeiodination for radiolabelling peptides of different biological importance. Gliadin-19 and Gliadin-33 with a molecular weight (as the trifluoroacetate salt) of 2591 and 4026 respectively are natural occurring peptide fragments of gluten proteins. PACAP-38 with a molecular weight of 6271 is biologically important due to a putative mechanism for inducing headache<sup>1</sup>.

The tritium labelling reactions have been performed using identical reaction conditions<sup>2</sup> but with different amounts of precursor peptide, we here compare the results from these reactions. The reactions were performed on a tritium manifold system (RC TRITEC, Teufen, Switzerland). In a small round bottomed flask synthetic precursor peptide as TFA salt (0.6 – 2.2  $\mu$ mol) was loaded together with catalyst (10% Pd/C, 4-5 mg) and suspended in DMF (1 ml). After the employment of 8-10 Ci of tritium gas for 2 hours, the peptide was purified and analysed. The peptide recovery (PR%), specific activity (SA) and radiochemical yield (RCY) were then determined. Results from 5 independent reactions are summarised in the table below.

Peptide	MW (TFA)	Reaction Conditions				Crude		Purified		
		Peptide [mg]	$\mu$ mol	Pd [mg]	T <sub>2</sub> [Ci]	RCY [mCi]	RCP%	PR%	SA [Ci/mmol]	RCY [mCi]
Gliadin-19	2591	4.1	1.582	5.1	10.0	50.9	100	66%	28.8	30.0
Gliadin-19		5.5	2.123	5.3	10.0	53.5	100	63%	29.7	39.6
Gliadin-33	4026	6.2	1.540	5.1	10.3	33.3	81	84%	15.9	20.5
Gliadin-33		9.2	2.285	5.3	10.0	31.8	61	68%	13.7	21.4
PACAP-38	6271	4.0	0.638	4.1	8.25	22.6	85	60%	25.1	9.7

Table: Result from 5 tritiation reactions. MW is protonated trifluoroacetate salt of precursor peptide, tritiation of Gliadine-19 showed only one peak in the radiochromatogram, thus the RCP of 100%.

Great success using these reaction conditions and methodology was found, both for the radiochemical yield and the peptide recovery. Double iodinated precursors yielded products with approx. one tritium atom per molecule.

## References

- [1] M. Baun, M. H. F. Pedersen, J. Olesen, I. Jansen-Olesen, *Cephalalgia* **2012**; 32, (4), 337-345.
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<sup>†</sup> We would like to acknowledge IAEA for the fellowship of Aleš Marek at the Hevesy Laboratory during 6 months in 2012.